Amendments to the Specification:

PLEASE NOTE WITH RESPECT TO AMENDMENTS BELOW: Some of these amendments are made to correct small errors in the application as originally filed while others are made to correct small errors that arose in the application as published on May 10, 2007 as US Pub. No. 20070105795. (For example, "UC Box" of [0015] is changed herein back to –C Box-- as originally filed.) Applicants also note that, as filed, the patent application did not have paragraph numbers. The paragraph numbers referenced below also appear in US Pub. No. 20070105795.

Please amend the Abstract as follows:

A method is provided for making gene suppression agents to be used in eukaryotic cells by using a recombinant DNA construct containing at least one transcriptional unit compromising a transcriptional promoter, a template sequence for making a RNA molecule, and a transcriptional terminator. Mechanisms of the RNA mediated gene suppression include, but **are** not limited to, RNA interferences (RNAi). The use of the agents as tools for biomedical research as well as medicinal products is also disclosed.

Please amend the specification as follows as indicated by paragraph:

[0005] RNA interference (RNAi) is a process of sequence-specific, post-transcriptional gene silencing (PTGS) in animals and plants initiated by double-stranded RNA (dsRNA) that is homologous to the silenced gene. It is an evolutionarily conserved phenomenon and a multi-step process that involves generations of active siRNAs in vivo through the action of a mechanism that is not fully understood. RNAi has been used as a reverse genetic tool to study gene function in multiple model organisms, such as plants, Caenorhabditis elegants and Drosophila, where large dsRNAs efficiently induce gene-specific silencing. In mammalian cells dsRNAs, 30 base pairs or longer, can activate antiviral response, leading to the nonspecific degradation of RNA transcripts and **to** a general shutdown of host cell protein translation. As a result, the

long dsRNA is not a general method for silencing specific genes in mammalian cells. Recently, various siRNAs that were synthesized chemically or generated biologically using DNA templates and RNA polymerases have been used to down regulate expression of targeted genes in cultured mammalian cells. Among approaches used, it is highly desirable to use DNA constructs that contain promoters of transcriptions and templates for siRNAs to generate siRNAs in vivo and in vitro. Though several different promoters have been adapted in such DNA constructs, types of promoters used remain limited to, Type III RNA polymerase III (Pol III) promoters, such as the U6 promoter and the H1 promoter, and promoters that require viral RNA polymerases, such as the T7 promoter. The present invention provides methods and designs to produce gene expression suppression agents that greatly expand potential usages of siRNAs.

[0007] The promoter is any native or engineered transcription promoter. As examples of such promoters (not intended on being limiting), in one embodiment, the promoter is a Type I Pol III promoter, while in another embodiment, the promoter is a combination of Type I Pol III promoter elements and Type III **Pol** ~~**Po**~~ III promoter elements. In other embodiments other types of promoters are present.

[0008] The targeted region of siRNA is anywhere on a transcript of any sequence in eukaryotic or viral genomes. The terminator is any native or engineered sequence that terminates the transcription by **Pol** ~~**Po**~~ III or other types of RNA polymerases.

[0009] Such gene expression suppression agents are delivered into eukaryotic cells, including (but not **limited** ~~**limiting**~~ to) mammals, insects, worms and plants, with any routes, procedures or methods, such as (but not limited to), in vivo, in vitro, ex vivo, electroporations, transfections or viral vector transduction.

[0010] FIG. 1 is a schematic representation of the embodiment for generating siRNA in mammalian cells using vertebrate Type I **Pol** ~~**Po**~~ III promoters. Specifically, FIG. 1 is a schematic representation of strategy for generating siRNA in mammalian cells using vertebrate Type I Pol III promoters (5S rRNA gene promoter and others). "A

Box", "C Box", "D Box" and "IE" are Pol III promoter elements, "+1" is an initiation site of transcription, "Tn" is a termination site of the **Pol** ~~Po~~ III promoter transcript, and the arrow indicates the orientation of transcription. The siRNA template consists of sense, spacer, antisense and terminator sequences, and generates a hairpin dsRNA when expressed. "Sense" is a 17-23 nucleotide (nt) sense sequence that is identical to that of the target gene and is a template of one strand of the stem in the hairpin dsRNA. "Space" is a 4-15 nt sequence and is a template of the loop of the strand of the stem in the hairpin dsRNA. "Terminator" is the transcriptional termination signal of five thymidines (5 Ts).

[0011] **FIG. 2 is** ~~FIG. "is~~ a schematic representation of the embodiment for generating siRNA in mammalian cells using vertebrate Type III Pol III promoters (U6 gene promoter, H1 RNA gene promoter, Y1 gene promoter, Y3 gene promoter, RNase P gene promoter and others). DSE, distal sequence element of Pol III promoter: PSE, proximal sequence element of Pol III promoter; TATA, a promoter element; +1, initiation site of transcription; the arrow indicates the orientation of transcription; siRNA Template, a 43-66 nt engineered insert that is the template for generating a hairpin dsRNA against a target gene; Sense, a 17-23 nt sense sequence from the target gene, template of one strand of stem in the hairpin; Spacer, a 4-15 nt sequence, template of loop of the hairpin; Antisense, a 17-23 nt antisense sequence, template of the other strand of stem in hairpin; Terminator, the transcriptional termination signal of 5 thymidines (5 Ts). FIG. 3 is a schematic representation of the embodiment for generating siRNA in mammalian cells using an engineered Pol III promoter containing the elements in both Type I and Type III promoters. "DSE" is a distal sequence element of Type III Pol III promoter. "PSE" is a proximal sequence element of Type III Pol III promoter, **"TATA"** ~~UTATA"~~ is a Type III Pol III promoter element. "A Box, "C Box" and "IE" are Type I Pol III promoter elements. "+1" is an initiation site of transcription. "Tn" is a termination site of the Type III Pol III promoter transcript. The arrow indicates the orientation of transcription. The siRNA template consists of sense, spacer, antisense and terminator sequences, and generates a hairpin dsRNA when expressed. "Sense" is a 17-23 nt sense sequence that is identical to that of the target gene and is a template of one strand of the stem in the

hairpin dsRNA. "Spacer" is a 4-15 nt sequence and is a template of the loop of the hairpin dsRNA. "Antisense" is a 17-23 nt antisense sequence and is a template of the other strand of stem in hairpin dsRNA. "Terminator" is the transcriptional termination signal of five thymidines (5 Ts).

[0014] FIG. 6 is a schematic representation of the embodiment for generating multiple siRNAs in mammalian cells using a single multiple transcription unit construct. "Unit" is a transcription unit that contains a vertebrate Type I Pol III promoter and a siRNA template. "A Box", "C Box", "D Box" and "IE" are Pol III promoter elements. "+1" is an initiation site of transcription. "Tn" is a termination site of the Pol III promoter transcript. The arrow indicates the orientation of transcription. The structure of siRNA template consists of sense, spacer, antisense and terminator sequences, and is an engineered insert that is the template for generating a hairpin dsRNA against a target gene. "Sense" is a 17-23 nucleotide (nt) sense sequence that is identical to that of the target gene and is a template of one strand of the stem in the hairpin dsRNA. "Spacer" is a **4-15** ~~**4--15**~~ nt sequence and is a template of the loop of the hairpin dsRNA. "Antisense" is a 17-23 nt antisense sequence and is a template of the other strand of stem in hairpin dsRNA. "Terminator" is the transcriptional termination signal of five thymidines (5 Ts). The multiple siRNAs may target a single region on one gene, different regions on one gene, or one region on each of many genes.

[0015] The following detailed description is provided to aid those skilled in the art to use the present invention. It should not be viewed as defining limitations of this invention. The present invention is directed to selectively suppress expression of genes targeted within mammalian cells by making and using DNA constructs that contains RNA polymerase III (Pol III) transcription promoter elements, template sequences for siRNAs, which are to be transcribed in host cells, and a terminator sequence. The promoter is any native or engineered transcription promoter. In one embodiment, the promoter is a Type I Pol III promoter. The essential elements of Type I promoter, such as "A Box", "C Box", "D Box" and "IE" are included in the DNA construct. In this embodiment, siRNA template is arranged between the "D Box" and "A Box". **In** ~~**As in**~~

another embodiment, the promoter is a combination of Type I Pol III promoter elements and Type III Pol III promoter elements. In this embodiment, the essential elements of both types of promoters, such "A Box", **"C Box",** ~~**UC Box",**~~ and "IE" of Type I promoter, as well as "DSE", "PSE" and "TATA" of Type III promoter are included in the DNA construct, with "DSE", "PSE" and "TATA" in the upstream region of "+1" position, "A Box", **"C Box",** ~~**UC Box",**~~ and "IE" in the down stream region of the "+1" position. Any promoter that is functioned in the mammalian cells is suitable to be used in this invention. Modifications, such as adding inducible or enhancing elements to exiting promoters, is suitable to be used in this invention.

[0017] The terminator is any native or engineered sequence that terminates the transcription by Pol III or other types of RNA polymerases, such as, **but** without **being** limited to, a stretch of 4 or more thymidines (T) residues in a DNA molecule.

[0027] The RNAi cassette will be synthesized as two strands and cloned between PstI and BbsI **sites** ~~**site**~~. The RNAi cassette is designed as follows: TABLE-US-00001 5' GC(N19)TTTCGG(61N)TTTTT 3' 3' ACGTCG(61N)AAAGCC(N19)AAAAATCGA 5'

[0030] ErbB2/Her2 gene is amplified **in about** ~~**in .about.**~~ 30% of breast cancers in **humans** ~~**human**~~, causing fast growth and metastasis of cancer cells. Herceptin, an antibody made by Genentech that blocks ErbB2 functions, is the only agent used by ErbB2-positive breast cancer patients that slows progression of metastatic breast disease and increases overall survival for patients given the drug along with standard chemotherapy compared to chemotherapy alone. Generation of siRNAs targeting ErbB2 developed with this invention should provide an alternative treatment.

[0031] Targeting BCR-Abl tyrosine kinase in chronic myelogenous leukemia (CML) and other cancers. BCR-Abl is a fusion gene product that frequently occurs in CML. ST1571, also called Gleevec developed by Novartis, is **a** newly approved anticancer agent to target BCR-Abl in CML. Generation of siRNAs against the fusion gene BCR-Abl, without interfering with the normal expression of either BCR or Abl gene, developed with this invention should have great potential for gene therapy to treat CML.

[0035] Using this invention to target different sites of the HIV genome will provide a potent gene therapy for HIV infected patients. A multiple units agent simultaneously targeting multiple sites, such as env, gag, pol, vif, nef, vpr, vpu and tat, may be suitable to address resistances resulted from mutations of **the** HIV genome.